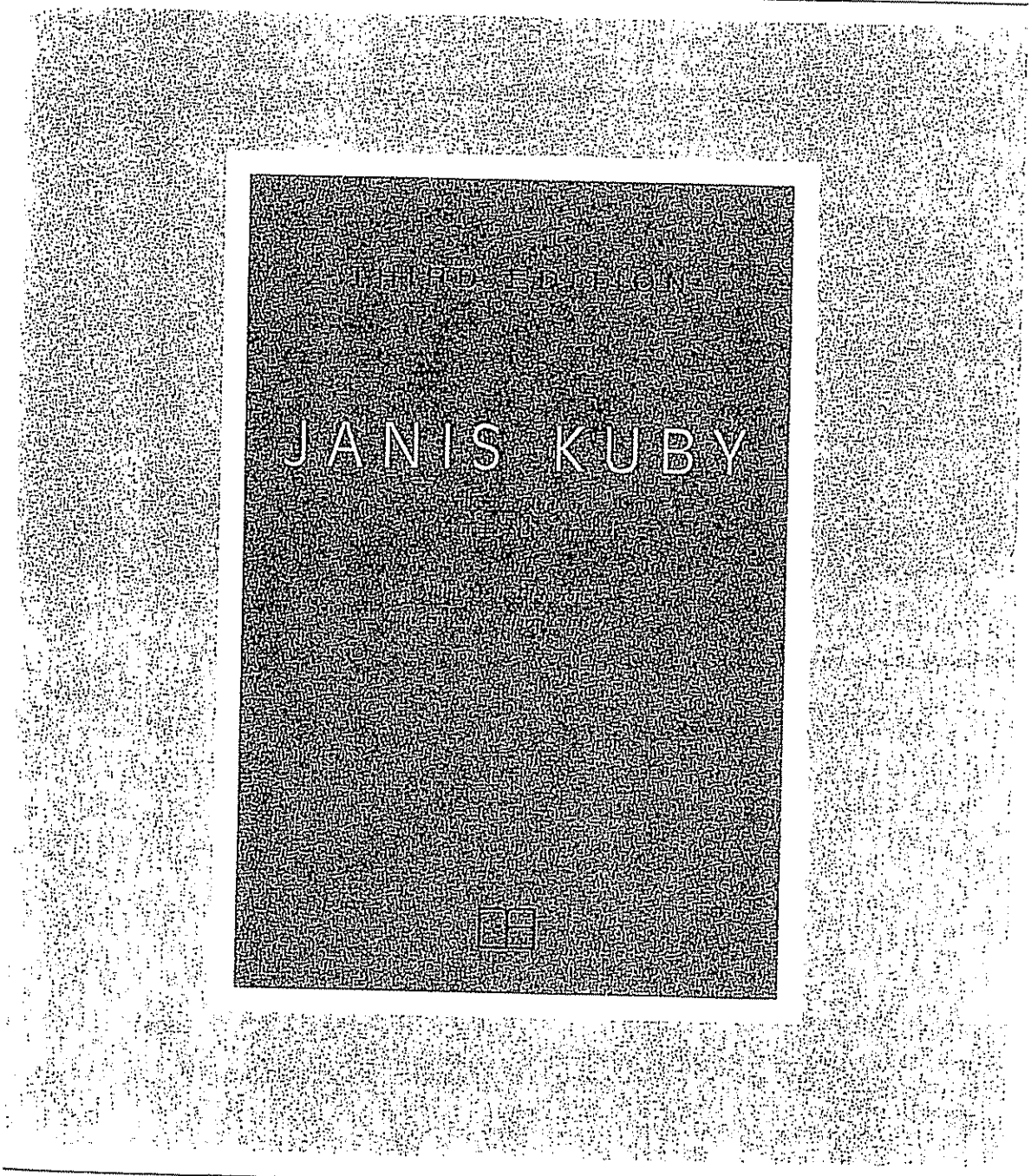
EXHIBIT 20

IMMUNOLOGY



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ABOUT THE COVER AND FRONTISPIECE

Interactions of cell adhesion molecules, with different ones involved at different times, are responsible for recruiting leukocytes to inflammatory sites and for their migration through the vascular endothelium. Slowed by vasodilation, leukocytes drift against vessel walls, where selectins are responsible for a loose adherence known as "rolling." This initial step in leukocyte migration is shown in a false-color scanning electron micrograph. (See Chapter 15 for more information.)

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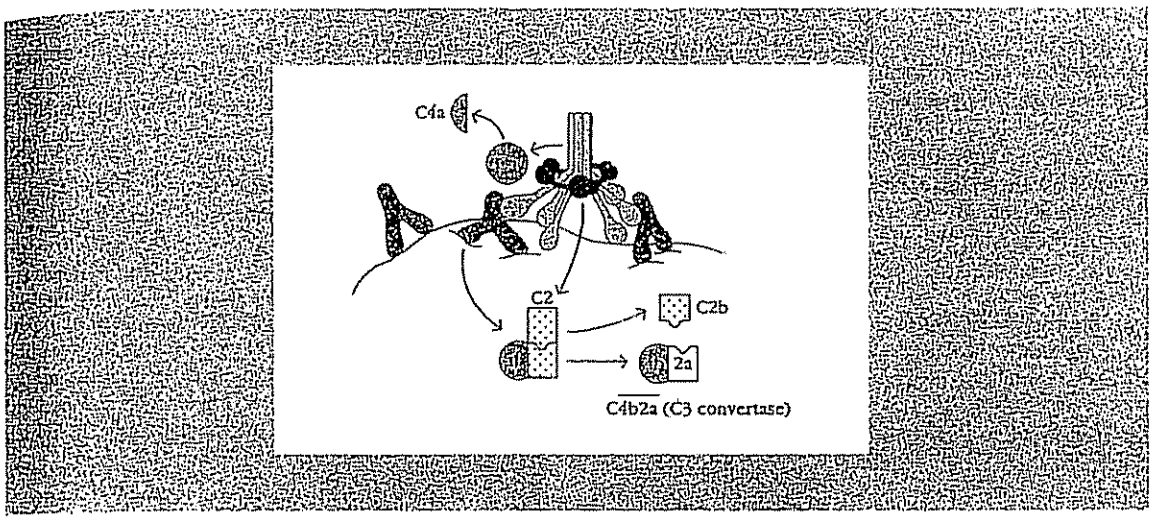
CHAPTER 14



THE COMPLEMENT SYSTEM

THE COMPLEMENT COMPONENTS
COMPLEMENT ACTIVATION
REGULATION OF THE COMPLEMENT SYSTEM
BIOLOGICAL CONSEQUENCES OF COMPLEMENT ACTIVATION
COMPLEMENT DEFICIENCIES

The complement system, the major effector of the humoral branch of the immune system, consists of nearly 30 serum and membrane proteins. Following initial activation, the various complement components interact, in a highly regulated enzymatic cascade, to generate reaction products that facilitate antigen clearance and generation of an inflammatory response. There are two pathways of complement activation: the classical pathway and the alternative pathway. The two pathways share a common terminal reaction sequence that generates a macromolecular membrane-attack complex (MAC), which lyses a variety of cells, bacteria, and viruses.

The complement reaction products amplify the initial antigen–antibody reaction and convert that reaction into a more effective defense mechanism. A variety of small, diffusible reaction products that are released during complement activation induce localized vasodilation and attract phagocytic cells chemotactically, leading to an inflammatory reaction. As antigen becomes coated with complement reaction products, it is more readily phagocytosed by phagocytic cells that bear receptors for these complement products. In addition, some of the complement products have been shown to play a role in the activation of B lymphocytes. Finally, the terminal components of the complement system generate the membrane-attack complex.

This chapter describes the similarities and differences in the two pathways, the regulation of the complement system, the effector functions of various complement components, and the consequences of hereditary deficiencies in some components.

THE COMPLEMENT COMPONENTS

The proteins and glycoproteins composing the complement system are synthesized largely by liver hepatocytes, although significant amounts of complement components are also produced by blood monocytes, tissue

macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts. These components constitute 15% (by weight) of the serum globulin fraction and circulate in the serum in functionally inactive forms, many of them as proenzymes in which the enzymatically active site is masked. Each proenzyme is activated by cleavage of the molecule, thereby removing an inhibitory fragment and exposing the active site. Activation of the complement system involves a sequential enzyme cascade in which the proenzyme product of one step becomes the enzyme catalyst of the next step. Each activated component has a short half-life before being inactivated.

Each complement component is designated by numerals (C1–C9), by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). The peptide fragments formed by activation of a component are denoted by small letters, with the smaller fragment designated "a" and the larger fragment designated "b" (e.g., C3a, C3b). The larger "b" fragments bind to the target near the site of activation, and the smaller "a" fragments diffuse from the site and play a role in initiating a localized inflammatory response. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., $C\overline{4b2a}$, $C\overline{3bBb}$).

COMPLEMENT ACTIVATION

The early steps in complement activation, culminating in formation of C5b, can occur via the **classical pathway** or the **alternative pathway**. The final steps leading to formation of a membrane-attack complex are the same in both pathways. The complement components involved in each pathway, and the sequence in which they take part, are outlined in Figure 14–1.

Classical Pathway

Complement activation via the classical pathway is commonly initiated by the formation of soluble antigen-antibody complexes (**immune complexes**) or by the

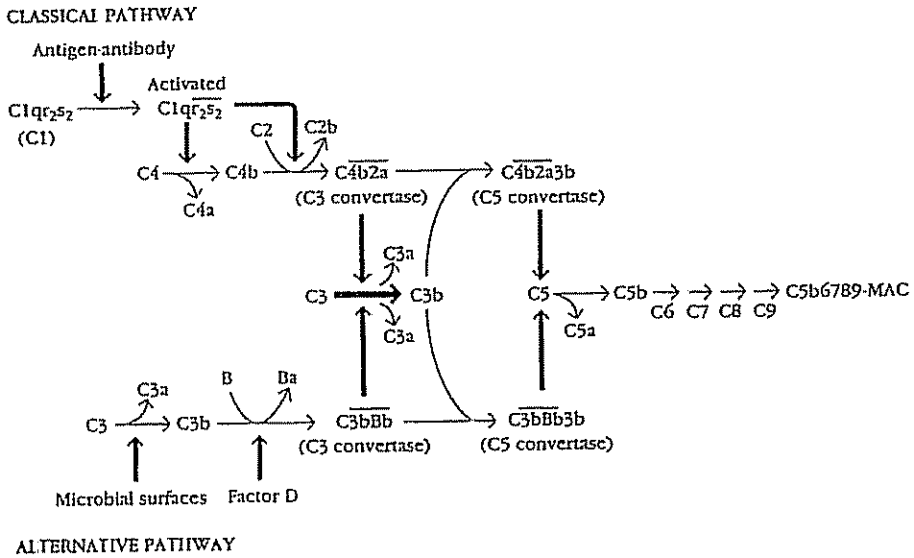


FIGURE 14-1

Overview of the complement activation pathways. The classical pathway is initiated by binding of C1 to antigen-antibody complexes. The alternative pathway is initiated by binding of C3b to activating surfaces such as microbial cell walls. Both pathways generate C3 and C5 convertases and bound C5b, which is converted into a membrane-attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in both pathways, generating large amounts of C3b, which forms part of C5 convertase. C3b also can diffuse away from the activating surface and bind to immune complexes or cell surfaces, where it functions as an opsonin. Blue arrows indicate reaction steps; black arrows indicate enzymatic or activating activity.

binding of antibody to antigen on a suitable target, such as a bacterial cell. IgM and certain subclasses of IgG (IgG1, IgG2, and IgG3) can activate the classical complement pathway, as can certain nonimmunologic activators. The initial stage of activation involves C1, C2, C3, and C4, which are present in plasma in functionally inactive forms (Table 14–1). The components were named in order of their discovery and before their functional roles had been determined, so that their names do not reflect the sequence in which they react.

The complexing of antibody with antigen induces conformational changes in the Fc portion of the antibody molecule that exposes a binding site for the C1 component of the complement system. C1 exists in serum as a macromolecular complex consisting of C1q and two molecules each of C1r and C1s, held together in a complex ($C1qr_2s_2$) stabilized by Ca^{2+} ions. The C1q molecule is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind to exposed C1q-binding sites in the C_H2 domain of the antibody molecule (Figure 14–2a,b). The $C1r_2s_2$ complex can exist in two configurations. When it is free and not bound to C1q, it assumes an S-shaped form; on binding to C1q, $C1r_2s_2$ assumes a shape similar to a figure 8 (Figure 14–2c,d,e). Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates interaction with C1q or with each other.

Each C1 molecule must bind, via its C1q globular heads, to at least two Fc sites for a stable C1–antibody interaction to occur. When pentameric IgM is bound to antigen on a target surface, at least three binding sites for C1q are exposed. Circulating IgM, however, assumes a planar configuration in which the C1q-binding sites are not exposed (Figure 14–3). For this reason, circulating IgM cannot activate the complement cascade by itself. An IgG molecule, on the other hand, contains only a single C1q-binding site in the C_H2 domain of the Fc, so that firm C1q binding is achieved only when two IgG molecules are within 30–40 nm of each other on a target surface or in a complex, providing two attachment sites for C1q. This difference in the structure of IgM and IgG accounts for the observation that a single molecule of IgM bound to a red blood cell is enough to activate the classical complement pathway and lyse the red blood cell, whereas some 1000 molecules of IgG are required if two molecules, randomly distributed, are to end up close enough to each other to initiate C1q binding.

The intermediates in the classical activation pathway are depicted schematically in Figure 14–4. Binding of C1q to Fc binding sites induces a conformational change in C1r that autocatalytically converts C1r to an active serine protease enzyme, $\overline{C1r}$, which then cleaves C1s to a similar active enzyme, $\overline{C1s}$. $\overline{C1s}$ has two substrates, C4 and C2 (see Figure 14–1). The C4 component is a glycoprotein containing three polypeptide chains (α, β, and γ). C4 is activated when $\overline{C1s}$ hydrolyzes a small fragment (C4a) from the amino terminus of the chain, exposing a binding site on the larger fragment (C4b). The C4b fragment attaches to the target surface in the vicinity of C1,

TABLE 14-1

CLASSICAL COMPLEMENT PATHWAY: PROTEINS THAT PARTICIPATE IN FORMATION OF C5 CONVERTASE

COMPONENT	ACTIVE PROTEIN/ SPLIT PRODUCT	IMMUNOLOGIC FUNCTION
C1	C1q	Binds to Fc region of IgM and IgG
	C1r	Serine protease; enzymatically activates C1s
	C1s	Serine protease; enzymatically activates C4 and C2
C4	C4a	Peptide mediator of inflammation (anaphylatoxin)
	C4b	Binds C2 forming complex that is cleaved by C1s to yield C4b2a
C2	C2a	Serine protease; C4b2a acts as C3 convertase
	C2b	Unknown function
C3	C3a	Peptide mediator of inflammation (anaphylatoxin)
	C3b	Binds to C4b2a to form C5 convertase; major opsonin

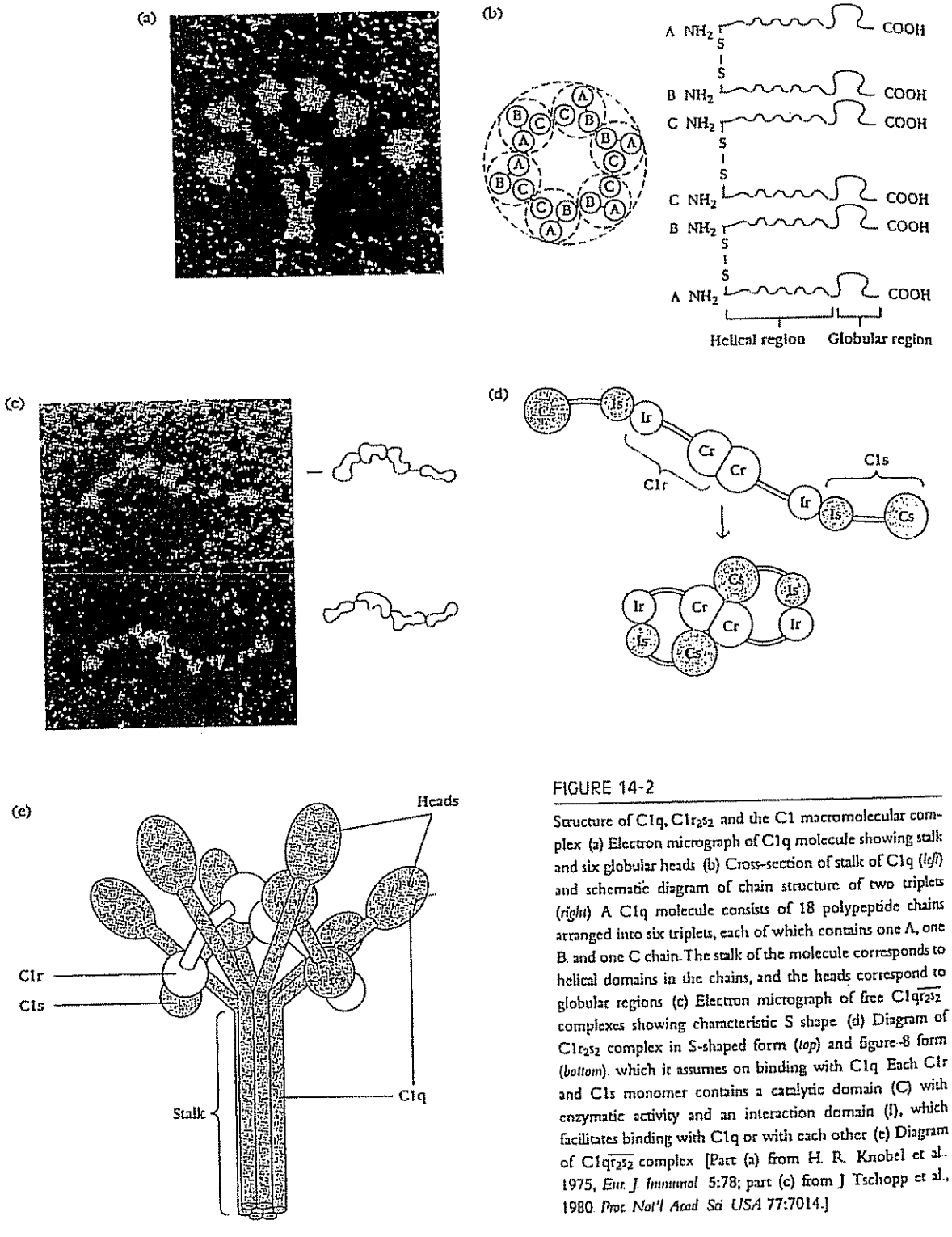


FIGURE 14-2

Structure of C1q, $C1r_2s_2$ and the C1 macromolecular complex (a) Electron micrograph of C1q molecule showing stalk and six globular heads (b) Cross-section of stalk of C1q (*left*) and schematic diagram of chain structure of two triplets (*right*) A C1q molecule consists of 18 polypeptide chains arranged into six triplets, each of which contains one A, one B and one C chain. The stalk of the molecule corresponds to helical domains in the chains, and the heads correspond to globular regions (c) Electron micrograph of free $C1q\overline{r_2s_2}$ complexes showing characteristic S shape (d) Diagram of $C1r_2s_2$ complex in S-shaped form (*top*) and figure-8 form (*bottom*), which it assumes on binding with C1q Each C1r and C1s monomer contains a catalytic domain (C) with enzymatic activity and an interaction domain (I), which facilitates binding with C1q or with each other (e) Diagram of $C1q\overline{r_2s_2}$ complex [Part (a) from H. R. Knobel et al., 1975, *Eur. J. Immunol.* 5:78; part (c) from J. Tschopp et al., 1980. *Proc. Nat'l. Acad. Sci. USA* 77:7014.]

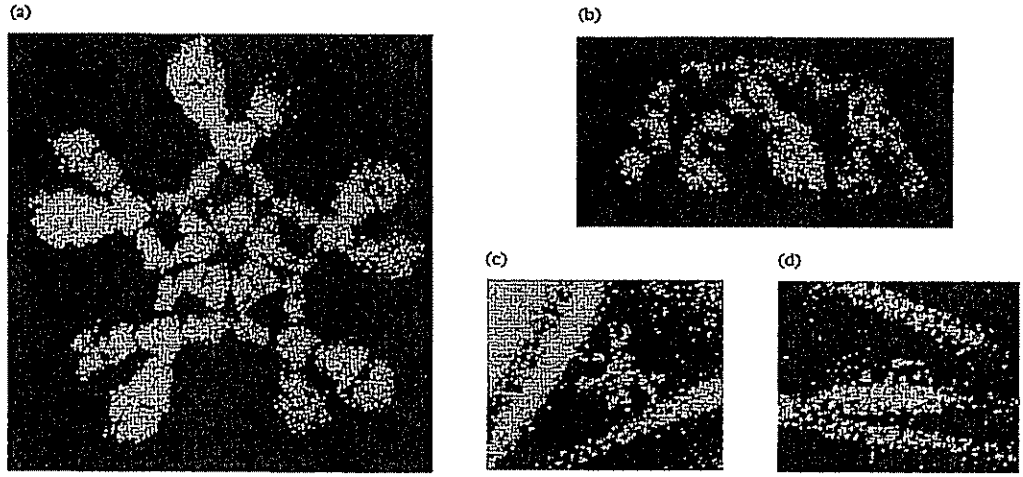


FIGURE 14-3

Models of pentameric IgM in planar form (a) and "staple" form (b). Several C1q-binding sites in the Fc region are accessible in the staple form, whereas none are exposed in the planar form. Electron micrographs of IgM antiflagellum antibody bound to flagella, showing the planar form (c) and stable form (d). [From A. Feinstein et al., 1981, *Monogr. Allergy* 17:28 and, 1981, *Ann. N.Y. Acad. Sci.* 190:1104.]

and the C2 proenzyme then attaches to the exposed binding site on C4b, where the C2 is then cleaved by the neighboring $\overline{C1s}$; the smaller fragment (C2b) diffuses away.* The resulting $\overline{C4b2a}$ complex is called **C3 convertase**, referring to its role in converting the C3 proenzyme into an enzymatically active form.

The native C3 component consists of two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) from the amino terminus of the α chain by the C3 convertase generates C3b (Figure 14–5). A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous **amplification** at this step of the sequence. Some of the C3b binds to $\overline{C4b2a}$ to form a trimolecular complex ($\overline{C4b2a3b}$) called **C5 convertase**. The C3b component of this complex binds C5 and alters its conformation, so that the $\overline{C4b2a}$ component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to the antigenic surface. The **bound C5b** initiates formation of the membrane-attack complex in a sequence described later. Some of the C3b generated by C3 convertase activity does not associate with $\overline{C4b2a}$; instead it diffuses away and then coats immune complexes and particulate antigens, functioning as an opsonin as discussed in a later section.

Alternative Pathway

Bound C5b can also be generated by the second major pathway of complement activation, the alternative pathway (see Figure 14–1). This pathway involves four serum proteins: **C3, factor B, factor D,** and **properdin** (Table 14–2). Unlike the classical pathway, which generally requires antibody to be initiated, the alternative pathway is initiated in most cases by various cell-surface constituents that are foreign to the host (Table 14–3). For example, both gram-negative and gram-positive bacteria have cell-wall constituents that can activate the alternative pathway. The intermediates in the alternative pathway for generating C5b are depicted schematically in Figure 14–6.

Serum C3, which contains an unstable thioester bond, is subject to slow spontaneous hydrolysis to yield C3a and C3b. The C3b component can bind to foreign surface antigens (such as those on bacterial cells or viral particles) or even to the host's own cells (see Figure 14–5c). The membranes of most mammalian cells have high levels of

* Contrary to the usual convention, the larger C2 fragment is designated C2a and the smaller fragment, C2b. Several years ago a proposal was made to reverse the C2 fragment designation so it would be similar to the other components; the first edition of this text adopted this proposal. However, this change in nomenclature ultimately was not approved, and subsequent editions of this text use the original nomenclature.

Visualizing Concepts

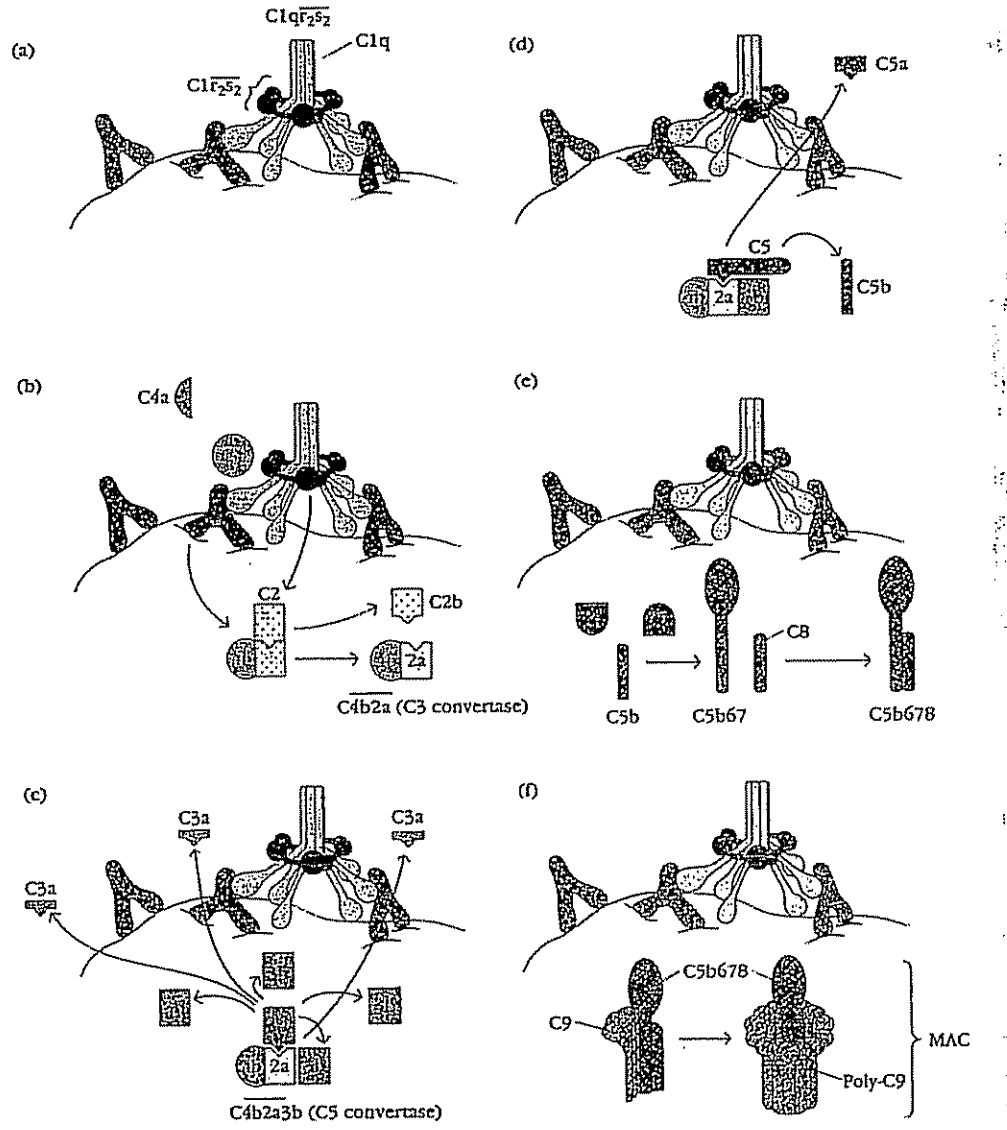


FIGURE 14-4

Schematic diagram of intermediates in the classical pathway of complement activation. Complement components shown in solid colors are bound to the antigenic surface but do not penetrate it; components that can insert into the cell membrane are marked with diagonal lines, and the freely diffusible components are stippled. The completed membrane attack complex (MAC) forms a large pore in the membrane. See text for details.

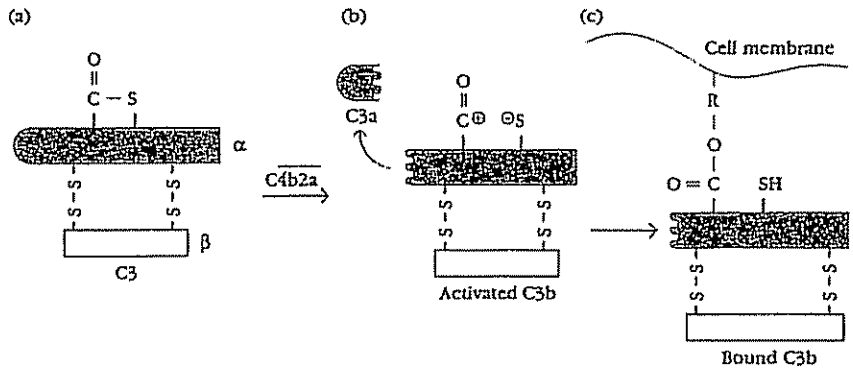


FIGURE 14-5

Hydrolysis of C3 by C3 convertase $\overline{C4b2a}$. (a) Native C3. (b) Activated C3 showing site of cleavage by $\overline{C4b2a}$, resulting in production of the C3a and C3b fragments. (c) A labile internal thioester bond in C3 is activated as C3b is formed, allowing the C3b fragment to bind to free hydroxyl or amino groups (R) on a cell membrane. Bound C3b exhibits various biological activities, including binding of C5 and binding to C3b receptors on phagocytic cells.

sialic acid, which contributes to the rapid inactivation of bound C3b molecules on host cells. Because many foreign antigenic surfaces (e.g., bacterial cell walls, yeast cell walls, and certain viral envelopes) have only low levels of sialic acid, C3b bound to these surfaces remains active for a longer time. Bound C3b can bind another serum protein called factor B by way of an Mg^{2+}-dependent bond. Binding to C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factor D. Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba), which diffuses away, and generating $\overline{C3bBb}$. The $\overline{C3bBb}$ complex has C3 convertase activity and thus is analogous to the $\overline{C4b2a}$ complex in the classical pathway (Table 14–4). The C3 convertase activity of $\overline{C3bBb}$ has a half-life of only 5 min unless the serum protein properdin binds to it, stabilizing it and extending the half-life of this convertase activity to 30 min.

The $\overline{C3bBb}$ generated in the alternative pathway can activate unhydrolyzed C3 to generate more C3b auto-

TABLE 14-2

ALTERNATIVE COMPLEMENT PATHWAY: PROTEINS THAT PARTICIPATE IN FORMATION OF C5 CONVERTASE

COMPONENT	ACTIVE PROTEIN/ SPLIT PRODUCT	IMMUNOLOGIC FUNCTION
C3	C3a	Peptide mediator of inflammation (anaphylatoxin)
	C3b	Binds factor B, forming complex that is cleaved by factor D to yield $\overline{C3bBb}$
Factor B	Ba	Unknown function
	Bb	Serine protease; $\overline{C3bBb}$ acts as C3 convertase, which generates $\overline{C3bBb3b}$ (C5 convertase)
Factor D	D	Serine protease; cleaves factor B that is bound to C3b to form C3 convertase
Properdin		Binds to and stabilizes $\overline{C3bBb}$

TABLE 14-3

INITIATORS OF THE ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN	NONPATHOGENS
Many strains of gram-negative bacteria	Human IgG, IgA, and IgE in complexes
Lipopolysaccharides from gram-negative bacteria	Rabbit and guinea pig IgG in complexes
Many strains of gram-positive bacteria	Cobra venom factor
Teichoic acid from gram-positive cell walls	Heterologous erythrocytes (rabbit, mouse, chicken)
Fungal and yeast cell walls (zymosan)	Anionic polymers (dextran sulfate)
Some viruses and virus-infected cells	Pure carbohydrates (agarose, inulin)
Some tumor cells (Raji)	
Parasites (trypanosomes)	

SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System*, Academic Press.

catalytically. As a result, the initial steps are repeated and amplified, so that more than 2×10^6 molecules of C3b can be deposited on an antigenic surface in less than 5 min. The C3 convertase activity of $\overline{C3bBb}$ generates the $\overline{C3bBb3b}$ complex, which exhibits C5 convertase activity, analogous to the $\overline{C4b2a3b}$ complex in the classical pathway. The nonenzymatic C3b component binds C5, and the $\overline{C3bBb}$ component subsequently hydrolyzes the bound C5 to generate C5a and C5b; the latter binds to the antigenic surface (see Figure 14-6).

Terminal Sequence: Formation of Membrane-Attack Complex

The terminal sequence of complement activation involves C5b, C6, C7, C8, and C9, which interact sequentially to form a macromolecular structure called the **membrane-attack complex**, or MAC (Table 14-5). This complex displaces the membrane phospholipids, forming a large transmembrane channel that disrupts the membrane and enables ions and small molecules to diffuse through it freely.

As noted previously, in both the classical and alternative pathways, a C5 convertase cleaves C5, which contains two protein chains (α and β). Following binding of C5 to the nonenzymatic C3b component of the convertase, the amino terminus of the α chain is cleaved, generating the small C5a fragment, which diffuses away, and the large C5b fragment, which provides a binding site for the subsequent components of the membrane-attack complex (see Figure 14-4d) The C5b component is extremely labile and is inactivated within 2 min unless C6 binds to it and stabilizes its activity.

Up to this point all the complement reactions take place on the hydrophilic surface of membranes or on immune complexes in the fluid phase. As C5b6 binds to C7, the resulting complex undergoes a hydrophilic-amphiphilic structural transition that exposes hydrophobic regions, which serve as binding sites for membrane phospholipids. If the reaction occurs on a target-cell membrane, the hydrophobic binding sites enable the C5b67 complex to insert into the phospholipid bilayer (see Figure 14-4e) If, however, the reaction occurs on an immune complex or other noncellular activating surface, then the hydrophobic binding sites cannot anchor the complex and it is released. Released C5b67 complexes can bind to nearby cells and mediate **"innocent-bystander" lysis**. In a number of diseases in which immune complexes are produced, tissue damage results from such innocent-bystander lysis. This autoimmune process will be discussed in Chapter 20.

Binding of C8 to membrane-bound C5b67 induces a conformational change in C8, so that it too undergoes a hydrophilic-amphiphilic structural transition exposing a

TABLE 14-4

COMPLEMENT COMPONENTS IN THE FORMATION OF C3 AND C5 CONVERTASES

	CLASSICAL PATHWAY		ALTERNATIVE PATHWAY	
Precursor proteins	C4	C2	C3	Factor B
Activating protease	C1s		Factor D	
C3 convertase	$\overline{C4b2a}$		$\overline{C3bBb}$	
C5 convertase	$\overline{C4b2a3b}$		$\overline{C3bBb3b}$	
C5-binding component	C3b		C3b	

Visualizing Concepts

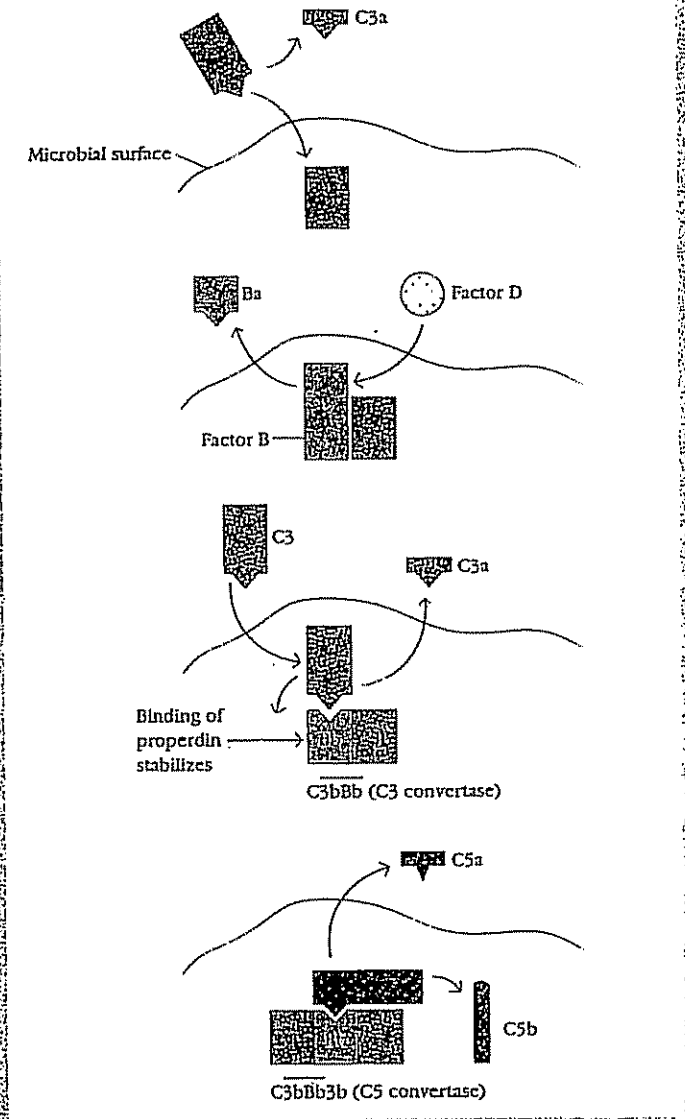


FIGURE 14-6 |

Schematic diagram of intermediates in formation of bound C5b by alternative pathway of complement activation. The C3bBb complex is stabilized by binding of properdin. Membrane-bound intermediates are shown in solid colors; components that can penetrate the cell membrane are marked with diagonal lines; freely diffusible components are supplied. Conversion of bound C5b to the membrane-attack complex occurs by the same sequence of reactions as in the classical pathway (see Figure 14–5). See text for details.

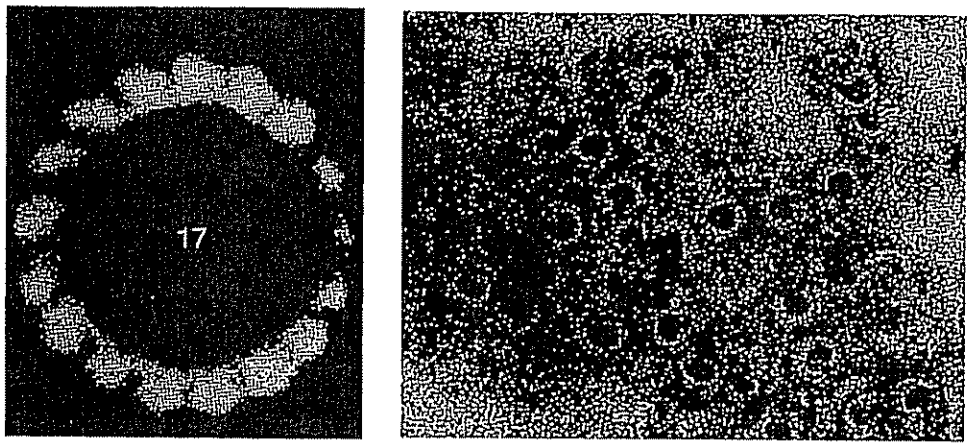


FIGURE 14-7

(a) Photomicrograph of poly-C9 complex formed by in vitro polymerization of C9. (b) Photomicrograph of complement-induced lesions on the membrane of a red blood cell. These lesions result from formation of membrane-attack complexes. [Part (a) from E. R. Podack, 1986, in *Immunobiology of the Complement System*. Academic Press; part (b) from J. Humphrey and R. Dourmashkin, 1969. *Adv. Immunol.* 11:75.]

hydrophobic region, which interacts with the plasma membrane. The C5b678 complex creates a small pore 10 Å in diameter; formation of this pore can lead to lysis of red blood cells but not of nucleated cells. The final step in formation of the MAC is the binding and polymerization of C9, a perforin-like molecule, to the C5b678 complex. As many as 10–16 molecules of C9 can be bound and polymerized by a single C5b678 complex. During polymerization the C9 molecules undergo a hydrophilic-amphiphilic transition, so that they also can insert into the membrane (see Figure 14-4f). The completed MAC, which has a tubular form and functional pore size of 70–100 Å, consists of a C5b678 complex surrounded by a **poly-C9 complex** (Figure 14-7). Since ions and small molecules can diffuse freely through the central channel of the MAC, the cell cannot maintain its osmotic stability and is lysed by an influx of water and loss of electrolytes.

TABLE 14-5

TERMINAL COMPLEMENT PATHWAY: PROTEINS INVOLVED IN THE FORMATION OF THE MEMBRANE-ATTACK COMPLEX (MAC)

COMPONENT	ACTIVE PROTEIN/ SPLIT PRODUCT	IMMUNOLOGIC FUNCTION
C5	C5a	Peptide mediator of inflammation (anaphylatoxin)
	C5b	Binds C6 to initiate formation of MAC
C6	C6	C5b6 binds C7
C7	C7	C5b67 binds C8; after an amphiphilic transition, the resulting complex inserts into the lipid bilayer
C8	C8	C5b678 binds multiple C9 molecules, initiating their polymerization
C9	C9	Polymerizes to complete formation of MAC pore

REGULATION OF THE COMPLEMENT SYSTEM

Because the complement system is nonspecific and thus capable of attacking host cells as well as microorganisms, elaborate regulatory mechanisms are required to confine the complement activation to designated targets. Both the classical and alternative pathways include a number of extremely labile components, which undergo spontaneous inactivation as they diffuse away from target cells. For example, the target-binding site on C3b undergoes spontaneous hydrolysis by the time it has diffused 40 nm away from the $\overline{C4b2a}$ or $\overline{C3bBb}$ convertase enzymes. This rapid hydrolysis limits binding of C3b to nearby host cells. In addition, both pathways include a series of regulatory proteins that inactivate various complement components (Table 14–6). For example, a glycoprotein called **C1 inhibitor** (C1Inh) can form a complex with $C1r_2s_2$, causing it to dissociate from C1q and preventing further activation of C4 or C2 (Figure 14–8a).

The reaction catalyzed by the C3 convertase enzymes of the classical and alternative pathways is the major amplification step in complement activation, generating hundreds of molecules of C3b. The C3b generated by these enzymes can bind to nearby cells, mediating damage to the healthy cells by opsonization to phagocytic

TABLE 14-6

PROTEINS THAT REGULATE COMPLEMENT SYSTEM

PROTEIN	TYPE OF PROTEIN	PATHWAY AFFECTED	IMMUNOLOGIC FUNCTION
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement receptor type 1 (CR1)*	Membrane bound	Classical & alternative	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I–catalyzed cleavage of C4b or C3b
Membrane cofactor protein (MCP)*	Membrane bound	Classical & alternative	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I–catalyzed cleavage of C4b or C3b
Decay-accelerating factor (DAF)*	Membrane bound	Classical & alternative	Accelerates dissociation of $\overline{C4b2a}$ and $\overline{C3bBb}$ (C3 convertases)
Factor I	Soluble	Classical & alternative	Serine protease. Cleaves C4b or C3b using C4bBP, CR1, factor H, DAF, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Membrane inhibitor of reactive lysis (MIRL)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	Soluble	Effector	Blocks anaphylatoxin activity of C3a, C4a, and C5a

* An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats

Visualizing Concepts

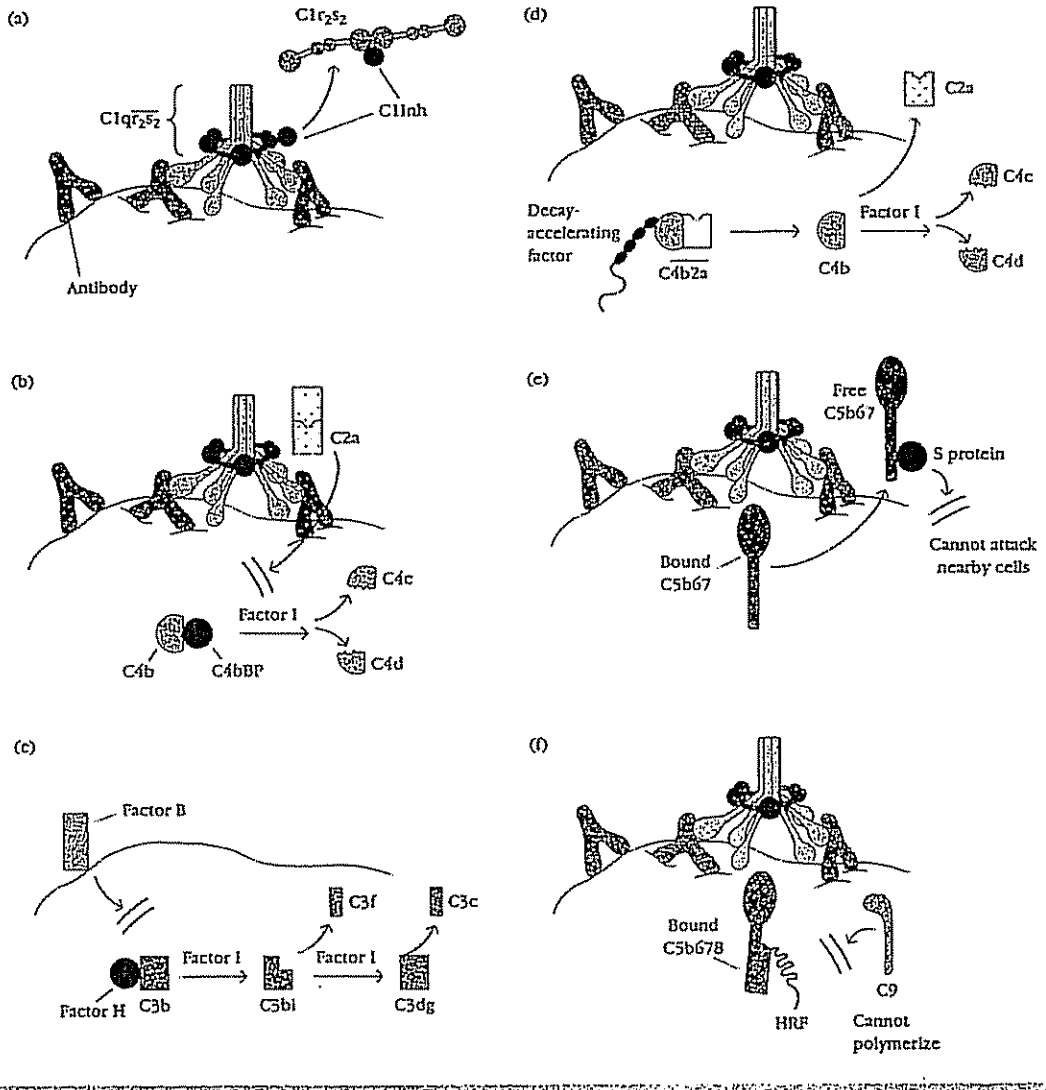


FIGURE 11-8

Schematic diagram of regulation of complement system by regulatory proteins (black) which either cause dissociation of activation intermediates or block their formation. Membrane-bound intermediates are shown in solid colors; components that can penetrate the cell membrane are marked with diagonal lines; freely diffusible components are supplied. C1inh = C1 inhibitor; C4bBP = C4b-binding protein; HRF = homologous restriction factor. See text and Table 9–6 for details.

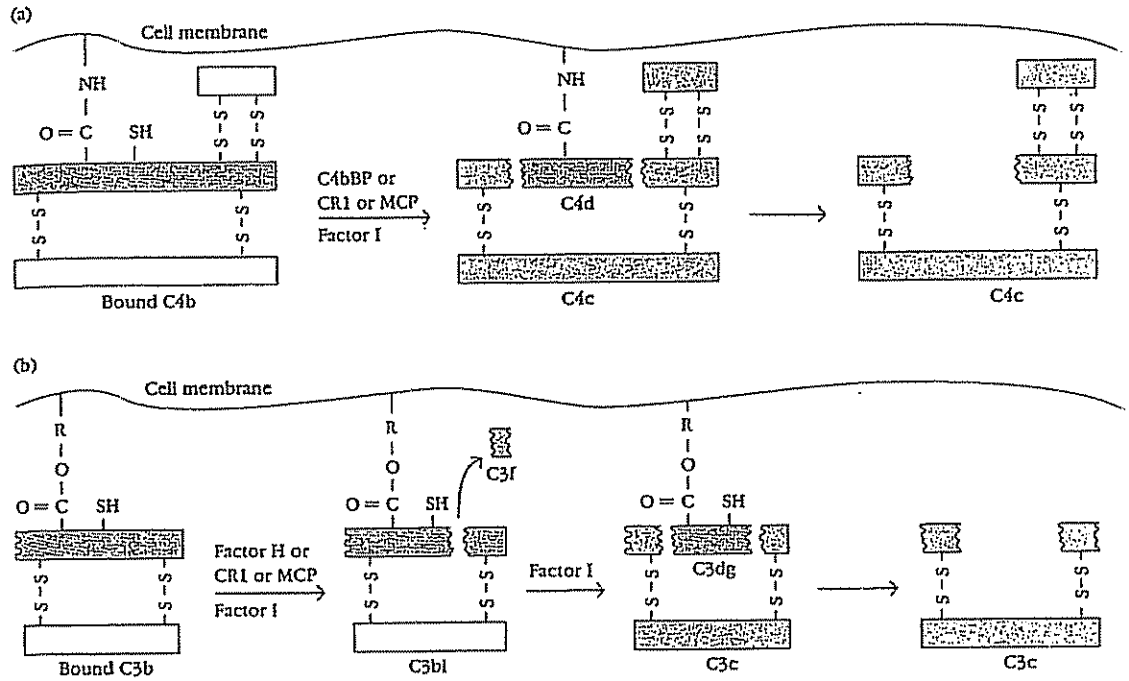


FIGURE 14-9

Inactivation of bound C4b and C3b by regulatory proteins of the complement system. (a) In the classical pathway, C4bBP (C4b-binding protein), CR1 (complement receptor type 1), or MCP (membrane cofactor protein) bind to C4b and act as cofactors for factor I–mediated cleavage of C4b. (b) In the alternative pathway, factor H, CR1, or MCP bind to C3b and act as cofactors for factor I–mediated cleavage of C3b. Free diffusible fragments are blue. See text for details.

cells bearing C3b receptors or by induction of the membrane-attack complex. It is estimated that circulating red blood cells are exposed to thousands of C3b molecules each day. C3b-mediated damage to healthy cells is prevented by a family of related proteins that regulate C3 convertase activity in the classical and alternative pathways. These C3 convertase regulatory proteins all contain repeating amino acid sequences (or motifs), containing about 60 residues, termed **short consensus repeats** (SCRs). All these proteins are encoded at a single chromosomal location on chromosome 1 in humans, known as the **regulators of complement activation** (RCA) gene cluster.

In the classical pathway three structurally different RCA proteins act similarly to prevent assembly of C3 convertase (Figure 14–8b). These regulatory proteins include soluble **C4b-binding protein** (C4bBP) and two membrane-bound proteins, **complement receptor type 1** (CR1) and **membrane cofactor protein** (MCP). Each of these regulatory proteins binds to C4b and prevents its association with C2a. Once C4bBP, CR1, or MCP is bound to C4b, another regulatory protein, **factor I**, cleaves the C4b into bound C4d and soluble C4c (Figure 14–9a). A similar regulatory sequence occurs in the alternative pathway. In this case CR1, MCP, or a regulatory component called **factor H** binds to C3b and prevents its association with factor B (Figure 14–8c). Once CR1, MCP, or factor H is bound to C3b, factor I cleaves the C3b into a bound C3bi fragment and a soluble C3f fragment. Further cleavage of C3bi by factor I releases C3c and leaves C3dg bound to the membrane (Figure 14–9b).

RCA proteins also act on the assembled C3 convertase, causing it to dissociate. Included among these regulatory proteins are the previously mentioned C4bBP, CR1, and factor H, as well as an additional protein, **decay-accelerating factor** (DAF). DAF, a glycoprotein, is anchored covalently to a glycophospholipid membrane

protein. Each of these RCA proteins accelerates decay (dissociation) of C3 convertase, releasing the component with enzymatic activity (C2a or Bb) from the cell-bound component (C4b or C3b). Once dissociation of the C3 convertase occurs, then factor I cleaves the remaining membrane-bound C4b or C3b component to irreversibly inactivate the convertase (Figure 14-8d).

Regulatory proteins also operate at the level of the membrane-attack complex. The ability of the C5b67 complex to be released and then bind to nearby cells poses a threat of innocent-bystander lysis of healthy cells. A number of serum proteins can counter this threat by binding to released C5b67 and preventing its insertion into the membrane of nearby cells. A serum protein called **S protein** can bind to C5b67, inducing a hydrophilic transition and thereby preventing insertion of C5b67 into the membrane of nearby cells (Figure 14-8e). The binding of the S protein to C5b67 also keeps C9 from binding to the soluble C5b67 and polymerizing, and thereby prevents the futile consumption of C9.

Complement-mediated lysis of cells is more effective if the complement is from a different species than the cells being lysed. This unusual phenomenon, which remained unexplained for several years, is now known to depend on two membrane proteins that block MAC formation. These two proteins, present on the membrane of many cell types, are **homologous restriction factor** (HRF) and **membrane inhibitor of reactive lysis** (MIRL). Both HRF and MIRL protect cells from nonspecific complement-mediated lysis by binding to C8, preventing assembly of poly-C9 and its insertion into the plasma membrane (Figure 14-8f). However, this inhibition occurs only if the complement components are from the same species as the target cells. For this reason, MIRL and HRF are said to display homologous restriction, for which the latter was named.

TABLE 14-7

SUMMARY OF BIOLOGICAL EFFECTS MEDIATED BY COMPLEMENT PRODUCTS

EFFECT	COMPLEMENT PRODUCT MEDIATING *
Cell lysis	**C5b–9, the membrane-attack complex (MAC)**
Inflammatory Response	
Degranulation of mast cells and basophils†	**C3a, C4a, and C5a (anaphylatoxins)**
Degranulation of eosinophils	**C3a, C5a**
Extravasation and chemotaxis of leukocytes at inflammatory site	**C3a, C5a, C5b67**
Aggregation of platelets	**C3a, C5a**
Inhibition of monocyte/macrophage migration and induction of their spreading	**Bb**
Release of neutrophils from bone marrow	**C3c**
Release of hydrolytic enzymes from neutrophils	**C5a**
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	**C5a**
Opsonization of particulate antigens, increasing their phagocytosis	**C3b, C4b, C3bi**
Viral neutralization	**C3b, C5b–9 (MAC)**
Solubilization and clearance of immune complexes	**C3b**

* Boldfaced component is most important in mediating indicated effect.

† Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

BIOLOGICAL CONSEQUENCES OF COMPLEMENT ACTIVATION

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms and viruses The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes (Table 14-7)

Many of the biological activities of the complement system depend on the binding of complement fragments to complement receptors, which are expressed by various cells In addition, some complement receptors play an important role in regulating complement activity by binding biologically active complement components and degrading them into inactive products. The complement receptors and their primary ligands, which include various complement components and their proteolytic breakdown products, are listed in Table 14-8.

Cell Lysis

The **membrane-attack complex** formed by complement activation is capable of lysing a broad spectrum of microorganisms, viruses, erythrocytes, and nucleated cells Because the alternative pathway of activation generally occurs without an initial antigen-antibody interaction, this pathway serves as an important innate system of nonspecific defense against infectious microorganisms. The requirement for an initial antigen-antibody reaction in the classical pathway supplements the nonspecific innate defense of the alternative pathway with a more specific defense mechanism.

The importance of cell-mediated immunity in host defense against viral infections has been emphasized in previous chapters Nevertheless antibody and complement do play a role in host defense against viruses and are often crucial in containing viral spread during acute

TABLE 14-8

COMPLEMENT-BINDING RECEPTORS

RECEPTOR	MAJOR LIGANDS	ACTIVITY	CELLULAR DISTRIBUTION
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,[a] C3bi	Part of B-cell coreceptor; binds Epstein-Barr virus	B cells, some T cells
CR3 (CD11b/18)	C3bi	Bind cell-adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis	Monocytes, macrophages, neutrophils, natural killer cells, some T cells
CR4 (CD11c/18)			
C3a/C4a receptor	C3a, C4a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes
C5a receptor	C5a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells

[a] Cleavage of C3dg by serum proteases generates C3d and C3g.

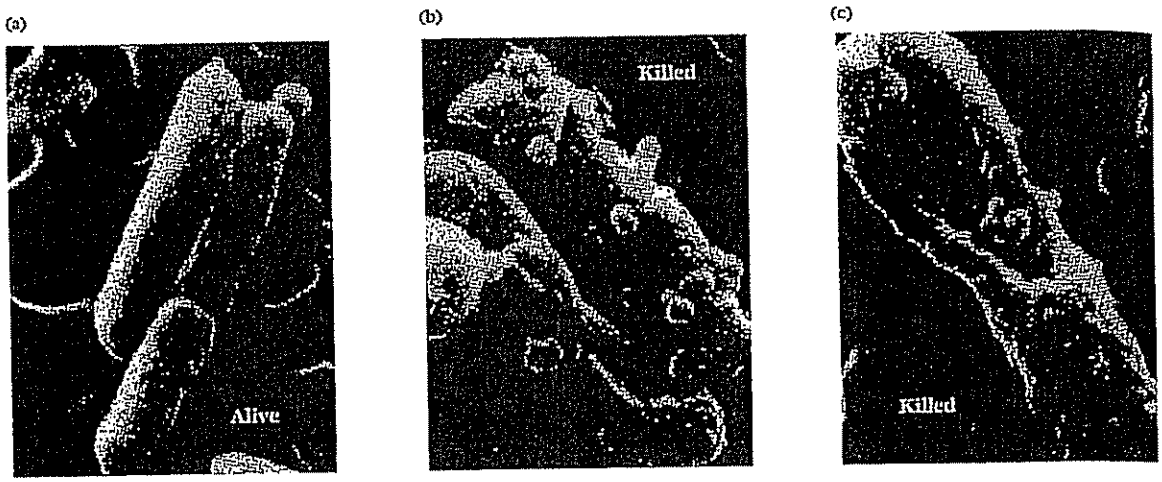


FIGURE 14-10

Scanning electron micrographs of *E. coli* showing (a) intact cells and (b, c) cells killed by complement-mediated lysis. Note membrane blebbing on lysed cells. [From R. D. Schreiber et al. 1979. *J. Exp. Med.* 149:870.]

infection and in protecting against reinfection. Most—perhaps all—enveloped viruses are susceptible to complement-mediated lysis. The viral envelope is largely derived from the plasma membrane of the infected host cell and is therefore susceptible to pore formation via the membrane-attack complex. Among the pathogenic viruses shown to be lysed by complement-mediated lysis are herpes virus, myxoviruses, paramyxoviruses, and retroviruses.

The complement system is generally quite effective in lysing gram-negative bacteria (Figure 14-10). However, some gram-negative bacteria and most gram-positive bacteria have mechanisms for evading complement-mediated damage (Table 14-9). For example, a few gram-negative bacteria can develop resistance to complement-mediated lysis that correlates with the virulence of the organism. In *Escherichia coli* and *Salmonella*, resistance to complement is associated with the smooth bacterial phenotype, which is characterized by the presence of long polysaccharide side chains in the cell-wall lipopolysaccharide (LPS) component. It has been proposed that the increased LPS in the wall of resistant strains may prevent insertion of the MAC into the bacterial membrane, so that the complex is released from the bacterial cell rather than forming a pore. Strains of *Neisseria gonorrhoeae* resistant to complement-mediated killing have been associated with disseminated gonococcal infections in humans. Some evidence suggests that the membrane proteins of resistant *Neisseria* strains undergo noncovalent interactions with the MAC that prevent its insertion into the outer membrane of the bacterial cells. These examples of resistant gram-negative bacteria are the exception; most gram-negative bacteria are susceptible to complement-mediated lysis.

In contrast, gram-positive bacteria are generally resistant to complement-mediated lysis because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane. Although complement activation can occur on the cell membrane of encapsulated bacteria such as *Streptococcus pneumoniae*, the capsule prevents interaction between C3b deposited on the membrane and the CR1 on phagocytic cells. Some bacteria possess an elastase that inactivates C3a and C5a, preventing these split products from inducing an inflammatory response. In addition to these mechanisms of evasion, various bacteria, viruses, fungi, and protozoans contain proteins that can interrupt the complement cascade on their surfaces, thus mimicking the effects of the normal complement regulatory proteins C4bBP, CR1, and DAF.

Nucleated cells tend to be more resistant to complement-mediated lysis than red blood cells. Lysis of nucleated cells requires formation of multiple membrane-attack complexes, whereas a single MAC can lyse a red blood cell. Many nucleated cells, including the majority of cancer cells, can endocytose the MAC. If the complex is removed soon enough, the cell can repair any membrane damage and restore its osmotic stability. This is the reason why complement-mediated lysis by monoclonal

antibody specific for tumor-cell antigens is often not effective; rather, such monoclonal antibodies must be conjugated with toxins or radioactive isotopes to be effective tumor-killing agents

Inflammatory Response

The complement cascade is often viewed in terms of the final outcome of cell lysis, but various peptides generated during formation of the MAC play a decisive role in the development of an effective inflammatory response (see Table 14–7). As noted already, the complement "split products" C3a, C4a, and C5a, called **anaphylatoxins**, bind to receptors on mast cells and blood basophils and induce degranulation with release of histamine and other pharmacologically active mediators. These mediators induce smooth-muscle contraction and increased vascular permeability. A serum protein called **anaphylatoxin inactivator** can bind C3a, C4a, and C5a, blocking their anaphylatoxin activity.

C3a, C5a, and C5b67 act together to induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues. C5a is most potent in mediating these processes, with picomolar quantities being effective. Activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry. The role of complement in the inflammatory response is discussed more fully in Chapter 15.

TABLE 14-9

MICROBIAL EVASION OF COMPLEMENT-MEDIATED DAMAGE

MICROBIAL COMPONENT	MECHANISM OF EVASION	EXAMPLES
	GRAM-NEGATIVE BACTERIA	
Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC in bacterial membrane	Resistant strains of *E. coli* and *Salmonella* sp.
Outer-membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylotoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	GRAM-POSITIVE BACTERIA	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus* sp.
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR 1 on phagocytic cells	*Streptococcus pneumoniae*
	OTHER MICROBES	
Proteins that mimic complement regulatory proteins	Proteins present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, *Trypanosoma cruzi*, *Candida albicans*

KEY: CR 1 = type 1 complement receptor; LPS = lipopolysaccharide; MAC = membrane-attack complex (C5b–9).

Opsonization of Antigen

C3b is the major **opsonin** of the complement system, although C4b and C3bi also have opsonizing activity. The amplification that occurs with C3 activation results in a coating of C3b on immune complexes and particulate antigens. Each of the phagocytic cells expresses complement receptors (CR1, CR3, and CR4) that bind C3b, C4b, or C3bi (see Table 14-8). When antigen has been coated with C3b during complement activation by either pathway, the coated antigen binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), phagocytosis will be enhanced (Figure 14-11).

Activation of phagocytic cells by various agents, including C5a anaphylatoxin, has been shown to increase the number of CR1s from 5000 on resting phagocytes to 50,000 on activated cells, greatly facilitating their phagocytosis of C3b-coated antigen. Once C3b-coated antigen has bound to CR1, some of the C3b is degraded into C3bi and C3f. This enables the antigen to bind to CR3, which triggers phagocytosis more effectively than CR1.

Viral Neutralization

The complement system plays an important role in host defense by neutralizing viral infectivity. Some viruses (e.g., retroviruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative or even the classical pathway in the absence of antibody. For most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by the classical pathway.

The complement system mediates viral neutralization by a number of mechanisms. Some degree of neutralization is achieved through the formation of larger viral aggregates, simply because these aggregates reduce the net number of infectious viral particles. Although antibody does play a role in the formation of viral aggregates, in vitro studies show that the C3b component facilitates aggregate formation in the presence of as little as two molecules of antibody per virion. For example, polyoma virus coated with antibody is neutralized when serum containing activated C3 is added.

The binding of antibody and/or complement to the surface of a viral particle creates a thick protein coating that can be visualized by electron microscopy (Figure 14-12). This coating neutralizes viral infectivity by blocking attachment to susceptible host cells. The deposits of antibody and complement on viral particles also facilitate binding of the viral particle to cells possessing Fc or type 1 complement receptors (CR1). In the case of phagocytic cells, such binding can be followed by phagocytosis and intracellular destruction of the ingested viral particle. Finally, complement is effective in lysing most, if not all, enveloped viruses, resulting in fragmentation of the envelope and disintegration of the nucleocapsid.

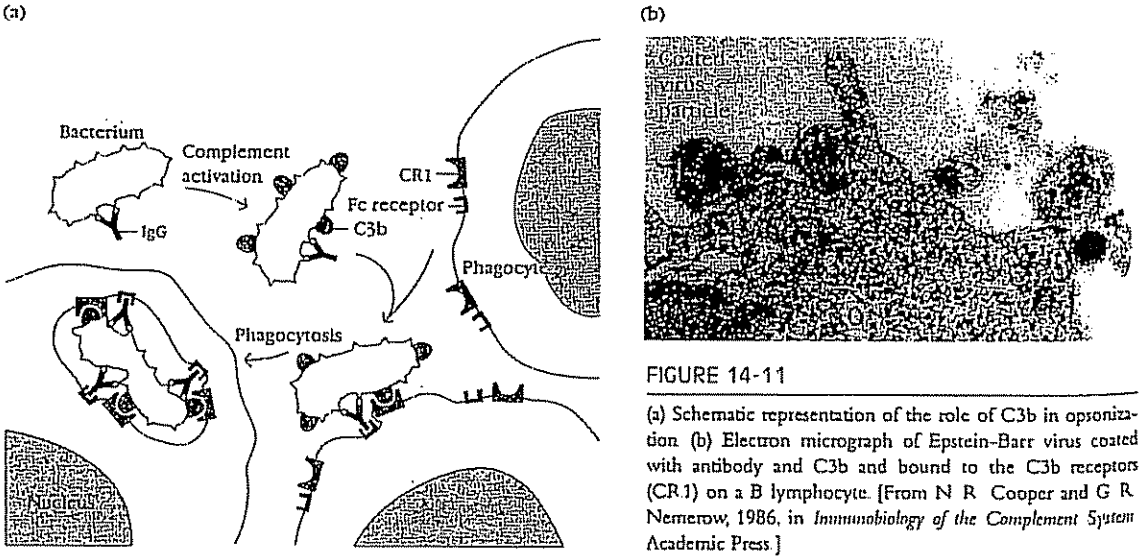


FIGURE 14-11

(a) Schematic representation of the role of C3b in opsonization. (b) Electron micrograph of Epstein-Barr virus coated with antibody and C3b and bound to the C3b receptors (CR1) on a B lymphocyte. [From N. R. Cooper and G. R. Nemerow, 1986, in *Immunobiology of the Complement System*. Academic Press.]

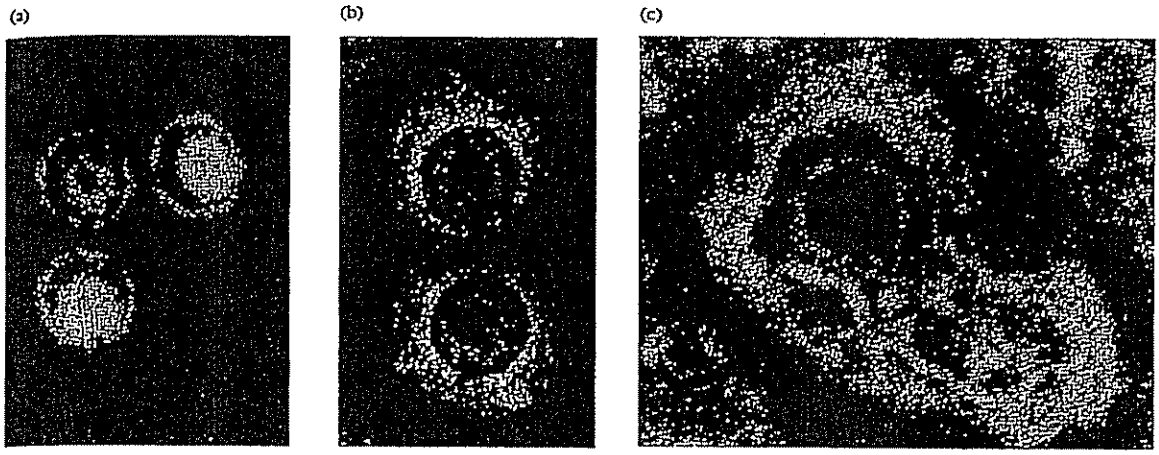


FIGURE 14-12

Electron micrographs of negatively stained preparations of Epstein-Barr virus (a) Control without antibody (b) Antibody-coated particles (c) Particles coated with antibody and complement [From N R Cooper and G R Nemerow, 1986, in *Immunobiology of the Complement System*. Academic Press]

Solubilization of Immune Complexes

The role of the complement system in clearing immune complexes can be seen in patients with the autoimmune disease **systemic lupus erythematosus** (SLE). These individuals produce large quantities of immune complexes and suffer tissue damage as a result of complement-mediated lysis and the induction of type II or type III hypersensitivity (see Chapter 17). Although complement plays a significant role in the development of tissue damage in SLE, the paradoxical finding is that deficiencies in C1, C2, C4, and CR1 predispose an individual to SLE; indeed, 90% of individuals who completely lack C4 develop SLE. The complement deficiencies are thought to interfere with effective solubilization and clearance of immune complexes; the result is the persistence of these complexes and subsequent tissue damage by the very system whose deficiency was to blame.

The coating of soluble immune complexes with C3b is thought to facilitate their binding to CR1 on erythrocytes. Although red blood cells express lower levels of CR1 ($\sim 5 \times 10^2$ per cell) than granulocytes ($\sim 5 \times 10^4$ per cell), there are about 10^3 red blood cells for every white blood cell; therefore, erythrocytes account for about 90% of the CR1 in the blood. For this reason, erythrocytes play an important role in binding C3b-coated immune complexes and carrying these complexes to the liver and spleen. In these organs, immune complexes are stripped from the red blood cells and are phagocytosed, thereby preventing their deposition in tissues. In SLE patients, deficiencies in C1, C2, and C4 each contribute to reduced levels of C3b on immune complexes and hence inhibit their clearance. The lower levels of CR1 expressed on the erythrocytes of SLE patients also may interfere with the proper binding and clearance of immune complexes

COMPLEMENT DEFICIENCIES

Genetic deficiencies have been described for each of the complement components with the exception of factor B. Homozygous deficiencies in any of the early components of the classical pathway (C1q, C1r, C1s, C4, and C2) manifest similar clinical presentations, notably a marked increase in **immune-complex diseases** such as systemic lupus erythematosus, glomerulonephritis, and vasculitis. These deficiencies highlight the important role of the early complement reactions in generating C3b, which is critical for solubilization and clearance of immune complexes. In addition to immune-complex diseases, individuals with such complement deficiencies may suffer from recurrent infections by pyogenic bacteria such as streptococci and staphylococci. These organisms are gram-positive and therefore resistant in any case to the lytic effects of the MAC. Nonetheless, the early

complement components ordinarily prevent recurrent infection by mediating a localized inflammatory response and opsonizing the bacteria. Deficiencies in factor D and properdin—early components of the alternative pathway—appear to be associated with *Neisseria* infections but not with immune-complex disease.

C3 deficiencies have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC. The first patient identified with a C3 deficiency was a child who suffered from frequent severe bacterial infections and was erroneously thought to have agammaglobulinemia. When tests revealed normal immunoglobulin levels, a deficiency in C3 was discovered. This case highlights the critical function of the complement system in converting a humoral antibody response into an effective host-defense mechanism. The majority of patients with C3 deficiency have recurrent bacterial infections and manifest immune-complex diseases.

Individuals with homozygous deficiencies in the components involved in the MAC develop recurrent meningococcal and gonococcal infections caused by *Neisseria* species. In normal individuals these gram-negative bacteria are generally susceptible to complement-mediated lysis or are cleared by the opsonizing activity of C3b. Few of these individuals manifest immune-complex disease, so generally they must produce enough C3b to clear immune complexes. Interestingly, a deficiency in C9 results in no clinical symptoms, suggesting that in some cases the entire MAC is not necessary for complement-mediated lysis to occur.

Congenital deficiencies of complement regulatory proteins have also been reported. The C1 inhibitor (C1Inh) regulates activation of the classical pathway by preventing excessive C4 and C2 activation by C1. Deficiency of C1Inh is an autosomal dominant condition with a frequency of 1 in 1000. The deficiency gives rise to a disease called **hereditary angioedema**, which manifests clinically as localized edema of the tissue, often following trauma but sometimes with no known cause. The edema can be in subcutaneous tissues or within the bowel or upper respiratory tract, where it causes abdominal pain or obstruction of the airway.

A number of the membrane-bound regulatory components including decay-accelerating factor (DAF) and homologous restriction factor (HRF) are anchored to the plasma membrane by glycosyl phosphatidylinositol membrane anchors. In individuals with **paroxysmal nocturnal hemoglobinuria** the glycosyl phosphatidylinositol membrane anchor is defective, resulting in an absence of DAF and HRF from the cell membrane. As a consequence of this defect, much lower levels of complement are able to lyse the red blood cells, and the individual suffers from chronic hemolytic anemia.

SUMMARY

1. The complement system comprises a group of serum proteins, many of which exist in inactive and active forms. Complement activation involves an enzymatic cascade that generates various complement proteins, which play an important role in antigen clearance. The two pathways of complement activation, the classical pathway and the alternative pathway, involve different complement proteins and are initiated differently (see Figure 14–1). The two pathways converge in a common terminal reaction sequence that generates a membrane-attack complex (MAC) responsible for cell lysis.
2. The classical pathway, which involves in order the C1, C4, C2, and C3 components, is initiated by binding of IgM and certain subclasses of IgG to soluble antigen or cell-surface antigens. The reaction sequence generates the enzymatically active $\overline{C4b2a}3b$ complex (C3 convertase), which can split C3 into C3a and C3b, and the $\overline{C4b2a3b}$ complex (C5 convertase), which can split C5 into C5a and C5b. The alternative pathway is most commonly initiated by surface constituents of a variety of microorganisms (bacteria, fungi, some viruses, and some parasites); however, this pathway also can be initiated by IgG-, IgA-, and IgE-antigen complexes (see Table 14–3). This pathway involves C3, factor B, factor D, and properdin. The reaction sequence generates $\overline{C3bBb}$ (C3 convertase) and $\overline{C3bBb3b}$ (C5 convertase) analogous to the convertases in the classical pathway. Both the classical and alternative pathways generate bound C5b. This component reacts sequentially with C6, C7, C8, and C9 to produce the membrane-attack complex, which mediates cell lysis by forming a large pore in the cell membrane (see Figures 14–4 and 14–6).
3. Because of its nonspecific nature, the complement system requires elaborate regulatory mechanisms to prevent damage to normal tissues. Both pathways have a number of extremely labile components that lose their activity as they diffuse from the site of activation. In addition, both pathways have a number of regulatory components that function to inactivate complement products and prevent excessive buildup of enzymatically active components (see Figure 14–8 and Table 14–6).
4. The complement system serves as an important effector of the humoral immune response with various components mediating specific effects (see Table 14–7). It destroys foreign cells through the process of MAC-mediated lysis. The complement system also induces a localized inflammatory response with a buildup of fluid and inflammatory cells, and it facili-

tates phagocytosis of antigen through its effect as an opsonin. Complement also acts to neutralize viral infectivity by several mechanisms and aids in solubilizing immune complexes.

5 Inherited deficiencies of most of the complement components have been described. The consequences of these conditions depend on which complement component is deficient. C3 deficiencies, which are clinically the most severe, are often associated with immune-complex disease and susceptibility to recurrent bacterial infections. These effects reflect the central role of C3 in both the classical and alternative pathways of complement activation. Deficiency of the regulatory protein C1 inhibitor (C1Inh) is fairly common and is associated with a localized edema called hereditary angioedema.

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STUDY QUESTIONS

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.
   a. A single molecule of bound IgM can activate the C1q component of the classical complement pathway.
   b. C3a and C3b are fragments of C3.
   c. The C4 and C2 complement components are present in the serum in a functionally inactive proenzyme form.
   d Nucleated cells tend to be more resistant to complement-mediated lysis than red blood cells.
   e Enveloped viruses cannot be lysed by complement because their outer envelope is resistant to pore formation by the membrane-attack complex.
   f. C4-deficient individuals have difficulty eliminating immune complexes.

2. Explain why serum IgM cannot activate complement by itself.

3. Would you expect a C1 or C3 complement deficiency to be more serious clinically? Why?

4 Some microorganisms produce enzymes that can degrade the Fc portion of antibody molecules. Why would such enzymes be advantageous for the survival of microorganisms that possess them?

5. Complement activation can occur via the classical or alternative pathway.
   a. How do the two pathways differ in the substances that can initiate activation?
   b. Which portion of the overall activation sequence differs in the two pathways? Which portion is similar?

c How do the biological consequences of complement activation via the classical and the alternate pathways differ?

6 Enucleated cells, such as red blood cells, are more susceptible to complement-mediated lysis than nucleated cells.

a. Explain why the red blood cells of an individual are not normally destroyed as the result of innocent-bystander lysis by complement

b. Under what conditions might complement cause lysis of an individual's own red blood cells?

7. Briefly explain the mechanism of action of the following complement regulatory proteins. Indicate which pathway(s) each protein regulates

a. C1 inhibitor (C1Inh)

b. C4b-binding protein (C4bBP)

c. Homologous restriction factor (HRF)

d. Decay-accelerating factor

e. Factor H

f. Membrane cofactor protein (MCP)

8 For each complement component(s) or reaction (a–l), select the most appropriate description listed below (1–13). Each description may be used once, more than once, or not at all.

Complement Component(s)/Reactions:

a. C3b

b C1, C4, C2, and C3

c. C9

d. C3, factor B, and factor D

e C1q

f $C\overline{4b2a3b}$

g. C5b, C6, C7, C8, and C9

h. C3 → C3a + C3b

i C3a, C5a, and C5b67

j C3a, C4a, and C5a

k $C\overline{4b2a}$

l C3b + B → $C\overline{3bBb}$ + Ba

Descriptions:

1) Reaction that produces major amplification during activation

2) Are early components of alternative pathway

3) Compose the membrane-attack complex

4) Mediates opsonization

5) Are early components of classical pathway

6) Has perforin-like activity

7) Binds to Fc region of antibodies

8) Have chemotactic activity

9) Has C3 convertase activity

10) Induce degranulation of mast cells (are anaphylatoxins)

11) Has C5 convertase activity

12) Reaction catalyzed by factor D

13) Reaction catalyzed by $Clq\overline{r_2s_2}$

9. You have prepared knockout mice with mutations in the genes that encode various complement components. Each knockout strain cannot express one of the complement components listed across the top of the table below. Predict the effect of each mutation on the steps in complement activation and on the complement effector functions indicated in the table below using the following symbols: NE = no effect; D = process/function decreased but not abolished; A = process/function abolished.

	Component knocked out						
	C1q	C4	C3	C5	C6	C9	Factor B
Complement Activation Formation of C3 convertase in classical pathway							
Formation of C3 convertase in alternative pathway							
Formation of C5 convertase in classical pathway							
Formation of C5 convertase in alternative pathway							
Effector Functions C3b-mediated opsonization							
Neutrophil chemotaxis							
Cell lysis							